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Developmental Evolution: Hox Proteins Ring the Changes

Dispatch

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The evolution of body form is believed to involve changes in expression of developmental genes, largely through changes in cis-regulatory elements. Recent studies suggest that changes in the sequences of key developmental regulators, such as the Hox proteins, may also play an important role.

The generation of morphological diversity in animals and plants is presumably based on changes in the developmental processes that control morphology. Comparative developmental studies have shown that many of these changes are regulatory, affecting the expression of developmental genes [1–4]. These can be achieved by changes in *cis*-regulatory elements or in the properties of *trans*-acting regulatory proteins — two types of change that are likely to have intrinsically different properties and evolutionary dynamics. Changes in *cis*-regulatory sequences are likely to have specific effects restricted to the expression of individual genes, while changes in regulatory proteins would be expected to affect the expression of multiple target genes, with potentially devastating effects.

Accordingly, *cis*-regulatory sequences show rapid evolutionary turnover [5], while regulatory proteins are often highly conserved in primary sequence and biochemical functions. For these reasons, changes in *cis*-regulatory elements are thought likely to play the larger part in morphological evolution. Two recent studies [6,7] now challenge this idea, suggesting that changes in important regulatory proteins, with multiple target genes, are also likely to play an important role in this process.

The two new studies [6,7] focus on one of the best understood interactions between a Hox protein and one of its targets: the regulation of *Distal-less* (*Dll*) by the Ultrabithorax (Ubx) protein in insects [8]. The insect body is typically subdivided into three regions: a head bearing sensory and feeding appendages (antennae and mouthparts); a thorax bearing locomotory appendages (legs and wings); and an abdomen that is typically devoid of limbs (Figure 1). Early expression of two *Hox* genes, *Ubx* and *Abdominal-A (AbdA)*, in the abdomen is responsible for the suppression of limbs in this body region. This is achieved, at least in part, by direct repression of the gene *Distal-less*, which is required for limb specification and growth.

In flies, Ubx and AbdA proteins are known to bind directly to an early enhancer of *Distal-less*, called *Dll-304*, and prevent it from activating *Distal-less* expression in abdominal segments [8,9]. This ability of Ubx and AbdA to repress *Distal-less* appears to have evolved specifically in the insect lineage, to create the limb-less abdominal region [10]. In crustaceans, the closest relatives of insects, Ubx and AbdA are produced in body regions that bear the most prominent and well-developed appendages (Figure 1), and are co-expressed with *Distal-less* throughout the development of appendages [1,11–13]. Thus, Ubx and AbdA proteins appear unable to repress *Distal-less* in these animals.

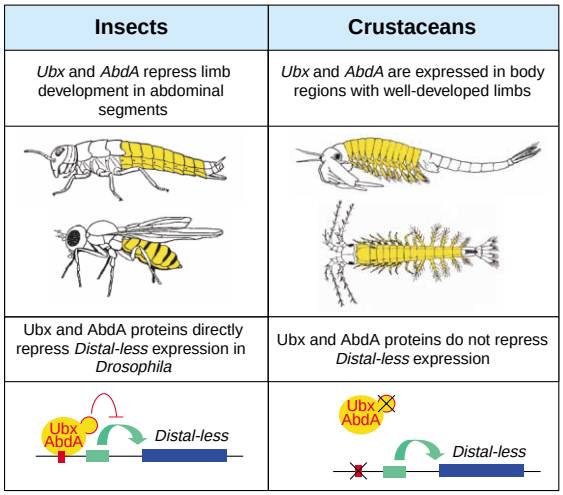

Figure 1. Different regulatory interactions between Hox proteins and a target gene.

In insects, *Ubx* and *AbdA* are expressed in the abdominal region (yellow), where they suppress the development of limbs. This is achieved by direct repression of the *Distal-less* gene. In crustaceans, *Ubx* and *AbdA* are expressed in regions of the trunk that bear well-developed appendages (yellow); *Distal-less* is not repressed, either because of differences in the crustacean Ubx and AbdA proteins (or their cofactors) that make them unable to exert this repression, or because of differences in the *Distal-less* cis-regulatory elements — such as the absence of Ubx/AbdA binding sites — that render the gene insensitive to this repression.

Is this difference the result of changes in the *cis*-regulatory elements of the *Distal-less* gene — for example, the presence or absence of Ubx/AbdA binding sites in a *Dll* enhancer — or of changes in the repressive ability of the Ubx and AbdA proteins themselves? Considering that these proteins are likely to regulate directly tens or hundreds of targets genes [14,15], changes in their properties could affect the expression of multiple genes, with dramatic consequences on morphology. The creation of simple Ubx/AbdA binding sites in the *Distal-less* enhancer would appear to be an easier and less hazardous way to achieve this change.

To approach this question, Ronshaugen *et al.* [6] and Galant and Carroll [7] cloned the *Ubx* homologues from different species and directly tested their ability to repress the *Dll-304* enhancer when expressed in

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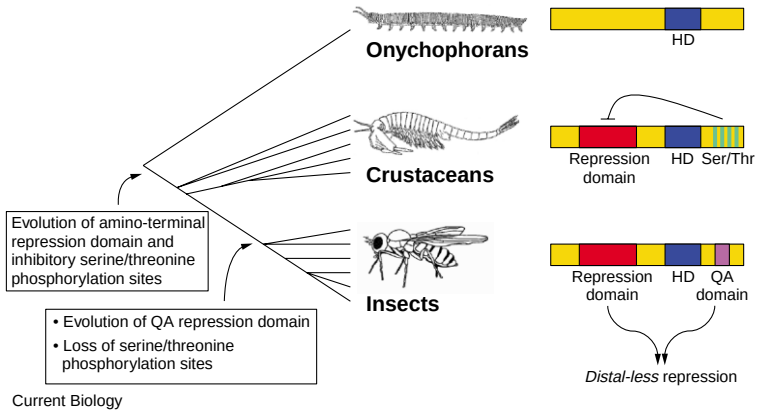

Figure 2. Changes in Ubx protein account for a change in the regulation of *Distal-less*.

The evolution of a QA repression domain and the loss of serine/threonine phosphorylation sites, which unmasks a repressive activity of the amino terminus of the protein, explain the repression of *Distal-less* and the evolution of a limb-less abdomen in insects.

flies. Galant and Carroll [7] looked in particular at the Ubx of an onychophoran, the most distant segmented relative of insects that still has a clearly recognisable Ubx homologue. In an earlier study [16] it was shown that onychophoran Ubx is unable to repress *Dll-304* in flies — although it is able to mimic other effects of the *Drosophila* Ubx protein — suggesting that some property of the protein has changed. Now, by testing the activity of chimeric Ubx proteins, where different domains have been swapped between the onychophoran and the *Drosophila* homologues, Galant and Carroll [7] identified a specific domain near the carboxyl terminus of *Drosophila* Ubx that is important for the repression of *Dll-304*. This so-called QA domain, consisting of a QAQAQK motif and a stretch of Ala residues, is conserved among insect Ubx proteins but absent or incomplete in other arthropod or onychophoran Ubx proteins. This domain is sufficient to confer repression activity when fused to onychophoran Ubx.

Ronshaugen *et al.* [6] went further, examining the Ubx protein of a crustacean. In similar domain-swapping experiments they found that, apart from the QA repression domain, insect Ubx has a second strongly repressive domain near its amino terminus. Strikingly, crustacean Ubx also has this amino-terminal repressive activity, but in crustaceans this is inhibited by a carboxy-terminal region that contains a series of putative serine/threonine phosphorylation sites (Figure 2). Mutation of the putative phosphorylation sites was found to generate a crustacean Ubx that strongly suppresses limbs.

Thus, insect Ubx proteins apparently acquired the ability to repress *Dll* not only by the evolution of the QA domain, but also by the gradual loss of phosphorylation sites that inhibit the intrinsic repressive ability of all — insect and crustacean — Ubx proteins (Figure 2). Interestingly, similar phosphorylation sites are also present in another Hox protein, Antp, which is produced in thoracic segments in insects. The presence of these sites in Antp prevents the protein from repressing *Dll* in the thorax [17], suggesting that this mechanism of controlling repression could be an ancient property shared by many Hox proteins.

These changes in the Ubx protein account very nicely for the evolution of *Distal-less* repression by Ubx and the suppression of limbs in the abdominal region of insects. Thus, a major morphological change, the creation of a limb-less abdominal region in insects, can be understood in terms of two simple steps in the evolution of Hox genes: the restriction of *Ubx* and *AbdA* expression to the posterior region of the trunk [11], and a change in the properties of their protein products that enables them to repress limbs [6,7]. This does not, of course, exclude the possibility that additional changes have taken place in the *cis*-regulatory elements of Hox target genes, but it does show that significant changes have taken place in the Ubx protein itself. An issue that remains open is what has happened to AbdA, the other Hox protein that represses *Distal-less* in the abdomen of insects (some studies suggest AbdA is the major player in this repression [9]). Has the AbdA protein, like Ubx, acquired new properties during the evolution of insects? Similar studies will have to be carried out on AbdA to answer this question.

As often happens in science, clever experiments raise new questions and force us to look at old problems in different ways. Crustacean Ubx has an amino-terminal repression domain that must presumably have a function when it is not inhibited by phosphorylation of the carboxy-terminal region. What are the circumstances in which this happens? More generally, how can a Hox protein have different repressive or activating effects in the context of different enhancers or different cell types [6,16,18]? Presumably different *cis*-regulatory elements can recruit different cofactors or activity modifiers, such as kinases, or some of these factors could be restricted to particular cell types. Bearing this in mind, can we be sure that *Drosophila* embryos and the *Drosophila Dll-304* enhancer provide the relevant context for assaying the activity of crustacean Ubx? To answer this question, one would love to be able to do the converse experiments in crustaceans, testing the activity of crustacean and insect Ubx proteins on a responsive *Dll* reporter. These questions apart, the papers of Ronshaugen *et al.* [6] and Galant and Carroll [7] provide an elegant example of how a regulatory change with important morphological consequences can be attributed to specific molecular changes in a regulatory protein.

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